

WHAT IS CLAIMED IS:

1. A method comprising:

providing spectrally resolved information about light coming from different
5 spatial locations in a sample comprising deep tissue in response to an illumination of the
sample, wherein the light includes contributions from different components in the sample;

decomposing the spectrally resolved information for each of at least some of the
different spatial locations into contributions from spectral estimates associated with at
least some of the components in the sample; and

10 constructing a deep tissue image of the sample based on the decomposition to
preferentially show a selected one of the components.

2. The method of claim 1, wherein the spectrally resolved information
comprises information about a set of images in which the light coming from the sample is
15 spectrally filtered, wherein the spectral filtering for each image corresponds to a different
spectral weighting function.

3. The method of claim 1, wherein the spectrally resolved information
comprises information about a set of images in which light used to illuminate the sample
20 is spectrally filtered, wherein the spectral filtering for each image corresponds to a
different spectral weighting function.

4. The method of claims 2 or 3, wherein the different spatial locations
correspond to common pixels in the set of images.

25 5. The method of claims 2 or 3, wherein the different spectral weighting
functions correspond to different spectral bands.

6. The method of claims 2 or 3, wherein the set of images comprises three or
30 more images.

7. The method of claims 2 or 3, wherein the set of images comprises four or
more images.

8. The method of claim 4, wherein the information about the set of images comprises a series of values at each of the pixels, wherein each value is related to an intensity of the light coming from the sample with respect to a corresponding one of the spectral weighting functions.

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9. The method of claim 1, wherein the spectrally resolved information for each spatial location comprises information corresponding to at least three different spectral weighting functions.

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10. The method of claim 1, wherein the spectrally resolved information for each spatial location comprises information corresponding to at least four different spectral weighting functions.

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11. The method of claim 1, wherein the spectrally resolved information comprises a spectral image cube.

12. The method of claim 1, wherein the light coming from the sample comprises fluorescence from the sample.

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13. The method of claim 1, wherein the light coming from the sample comprises reflectance, phosphorescence, scattering, or Raman scattering from the sample.

14. The method of claim 1, wherein the light coming from the sample comprises transmission through the sample.

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15. The method of claim 1, wherein at least one of the components relates to autofluorescence.

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16. The method of claim 1, wherein at least one of the components comprises a target compound.

17. The method of claim 16, wherein the selected component is the component comprising the target compound.

18. The method of claim 16, wherein the target compound comprises a fluorescent protein or a quantum dot.

19. The method of claim 1, further comprising illuminating the sample and
5 collecting the spectrally resolved information.

20. The method of claim 1, wherein collecting the spectrally resolved information comprises using a liquid crystal tunable spectral filter, an acousto-optical tunable spectral filter, a set of spectral filters, a spectral filter wheel, a dispersive prism, a
10 grating, a spectrometer, or monochromator.

21. The method of claim 1, wherein the deep tissue image of the selected component comprises an image in which signal from the other components is reduced relative to signal from the selected component.

15 22. The method of claim 1, further comprising constructing a second deep tissue image of the sample based on the decomposition to preferentially show a second one of the components.

20 23. The method of claim 1, further comprising constructing a third deep tissue image of the sample based on the decomposition to preferentially show a third one of the components.

24. The method of claim 1, wherein the sample is a living organism.

25 25. The method of claim 1, wherein the sample is a mammal.

26. The method of claim 1, wherein at least one of the spectral estimates is an estimate of the pure spectrum of a first one of the components.

30 27. The method of claim 26, wherein at least some of the spectral estimates are estimates of the pure spectra for some of the components.

28. The method of claim 26, wherein the first component corresponds to autofluorescence.

29. The method of claim 26, wherein the first component corresponds to the selected component.

30. The method of claim 26, wherein the pure spectrum for the first component corresponds to the spectrally resolved information that would result if only the first component contributes to the light.

31. The method of claim 1, wherein constructing the deep tissue image based on the decomposition comprises constructing the deep tissue image based on the contributions at different spatial locations of the spectral estimate associated with the selected component.

32. The method of claim 1, wherein the decomposition is a linear decomposition.

33. The method of claim 32, wherein the decomposition comprises solving at least one component of a matrix equation in which one matrix in the equation is based on the spectrally resolved information and another matrix in the equation is based on the spectral estimates.

34. The method of claim 1, wherein at least one of the spectral estimates is provided independently of the spectrally resolved information.

35. The method of claim 1, wherein at least a first one of the spectral estimates for a first one of the components is determined from the spectrally resolved information.

36. The method of claim 35, wherein all of the spectral estimates are determined from the spectrally resolved information.

37. The method of claim 36, wherein the spectral estimates are determined from the spectrally resolved information by using an unsupervised classification technique.

5 38. The method of claim 37, wherein the spectral estimates are determined from the spectrally resolved information by using a supervised classification technique.

39. The method of claim 37, wherein the unsupervised classification technique comprises averaging the spectrally resolved information for multiple ones of the spatial
10 locations.

40. The method of claim 35, wherein the first spectral estimate is determined from a region comprising one or more of the spatial locations, wherein the region is associated with the first component.

15 41. The method of claim 35, wherein the first spectral estimate is derived from the spectrally resolved information from a first set of one or more spatial locations in which the light includes contributions from multiple ones of the components.

20 42. The method of claim 41, wherein the first spectral estimate is derived from the spectrally resolved information from the first set of spatial locations and a second one of the spectral estimates for a second one of the components.

43. The method of claim 42, wherein deriving the first spectral estimate
25 comprises calculating a remainder spectrum based on the spectrally resolved information from the first set and the spectral estimate for the second component.

44. The method of claim 43, wherein the remainder spectrum is calculated at each of one or more of the spatial locations in the first set of spatial locations.

30 45. The method of claim 43, wherein the remainder spectrum is calculated based on an average of the spectrally resolved information in the first set of spatial locations and the spectral estimate for the second component.

46. The method of claim 42, wherein the spectral estimate for the second component is derived from the spectrally resolved information.

47. The method of claim 46, wherein the spectral estimate for the second
5 component is determined from the spectrally resolved information by using an unsupervised classification technique.

48. The method of claim 46, wherein the spectral estimate for the second component is derived from a region comprising one or more of the spatial locations,
10 wherein the region is associated with the second component.

49. The method of claim 42, wherein deriving the first spectral estimate comprises adjusting values corresponding to the spectrally resolved information for the first set of spatial locations to remove a contribution from the second component based on
15 the spectral estimate for the second component.

50. The method of claim 49, wherein the removed contribution is a maximal contribution.

20 51. The method of claim 50, wherein the maximal contribution is based on an error function analysis of signal in each spectral channel of the adjusted values.

52. The method of claim 51, wherein the error function analysis tends to maintain nonnegative signal in each spectral channel of the adjusted values.

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53. The method of claim 49, wherein the values comprises a series of at least some of the values for each of the spatial locations in the first set, and wherein removing the contribution from the second component based on the spectral estimate for the second component comprises subtracting an optimized quantity of the spectral estimate for the
30 second component from each of the series of values.

54. The method of claim 53, wherein determining the optimized quantity for at least a first of the series of values is based on minimizing an error function of a difference spectrum that includes a difference between the first series values and the quantity to be

optimized multiplied by the spectral estimate for the second component, wherein the error function is minimized over the first series of values.

55. The method of claim 54, wherein the difference spectrum further includes
5 a constant that is also optimized over the first series of values.

56. The method of claim 54, wherein the error function favors positive values of the difference spectrum over negative values of the difference spectrum.

10 57. The method of claim 56, wherein the error function comprises $(e^{-\Delta} + 1)\Delta^2$, where Δ is the difference spectrum.

58. The method of claim 54, wherein the error function is normalized by the magnitudes of the first series of values and the spectral estimate for the second
15 component.

59. The method of claim 35, wherein the decomposition comprises a first decomposition of the spectrally resolved information at multiple spatial locations into contributions from initial spectral estimates associated with at least some of the
20 components in the sample, improving an accuracy of at least some of the initial spectral estimates based on the first decomposition, and at least a second decomposition of the spectrally resolved information at multiple spatial locations into contributions from the improved spectral estimates.

25 60. A method comprising:
providing spectrally resolved information about light coming from different spatial locations of a sample comprising deep tissue in response to an illumination of the sample, wherein the light includes contributions from different components in the sample and the spectrally resolved information for each spatial location comprises information
30 corresponding to at least three different spectral weighting functions; and
constructing a deep tissue image of the sample based on the spectrally resolved information to preferentially show a selected one of the components.

61. The method of claim 60, wherein each spectral weighting function corresponds to a different spectral band.

62. The method of claim 60, wherein the spectrally resolved information for each spatial location comprises information corresponding to at least four different spectral weighting functions.

63. The method of claim 60, further comprising decomposing the spectrally resolved information for each of at least some of the different spatial locations into a contribution from a spectral estimate associated with at least one of the components in the sample.

64. The method of claim 63, wherein the spectral estimate is an estimate of the pure spectrum of a first one of the components.

65. The method of claim 64, wherein the pure spectrum for the first component corresponds to the spectrally resolved information that would result if only the first component contributes to the light.

66. The method of claim 63, wherein the decomposing comprises decomposing the spectrally resolved information for each of at least some of the different spatial locations into contributions from spectral estimates associated with at least some of the components in the sample.

67. The method of claim 66, further comprising constructing a second deep-tissue image of the sample based on the decomposition to preferentially show a second one of the components.

68. A method comprising:
providing spectrally resolved information about light coming from different spatial locations of a biological sample in response to an illumination of the sample, wherein the light includes contributions from different components in the sample;

decomposing the spectrally resolved information for each of at least some of the different spatial locations into a contribution from a spectral estimate of a pure spectrum for at least a first one of the components in the sample; and

constructing an image of the sample based on the decomposition to preferentially
5 show a selected one of the components,

wherein the decomposition comprises deriving the estimate of the pure spectrum for the first component based on both the spectrally resolved information corresponding to a first set of one or more of the different spatial locations and a spectral estimate of a pure spectrum for a second one of the components.

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69. The method of claim 68, wherein the sample comprises deep tissue, tissue slices, cells, subdermal tissue, or a microscope slide carrying biological material.

70. The method of claim 68, wherein the decomposing comprises
15 decomposing the spectrally resolved information for each of at least some of the different spatial locations into contributions from estimates of pure spectra for at least some of the components in the sample.

71. The method of claim 68, wherein the decomposition is a linear
20 decomposition.

72. The method of claim 71, wherein the decomposition comprises solving at least one component of a matrix equation in which one matrix is based on the spectrally resolved information and another matrix is based on the estimate of the pure spectrum for
25 the first component and an estimate for the pure spectrum of at least one additional component.

73. The method of claim 68, wherein the pure spectrum for the first component corresponds to the spectrally resolved information that would result if only the
30 first component contributes to the light.

74. The method of claim 68, wherein the spectral estimate of the pure spectrum for the second component is provided independently of the spectrally resolved information.

75. The method of claim 68, wherein the spectral estimate of the pure spectrum for the second component is determined from the spectrally resolved information.

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76. The method of claim 75, wherein the spectral estimate of the pure spectrum for the second component is determined from the spectrally resolved information by using an unsupervised classification technique.

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77. The method of claim 75, wherein the spectral estimate of the pure spectrum for the second component is determined from the spectrally resolved information by using a supervised classification technique.

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78. The method of claim 77, wherein the unsupervised classification technique comprises averaging the spectrally resolved information for multiple ones of the spatial locations.

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79. The method of claim 75, wherein the spectral estimate of the pure spectrum for the second component is determined from the spectrally resolved information by associated a region of one or more of the spatial locations with the second component.

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80. The method of claim 68, wherein the spatial locations in the first set are spatial location in which the light includes contributions from multiple ones of the components.

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81. The method of claim 68, wherein deriving the spectral estimate of the pure spectrum for the first component comprises calculating a remainder spectrum based on the spectrally resolved information from the first set and the spectral estimate for the second component.

82. The method of claim 81, wherein the remainder spectrum is calculated at each of one or more of the spatial locations in the first set of spatial locations.

83. The method of claim 82, wherein the remainder spectrum is calculated based on an average of the spectrally resolved information in the first set of spatial locations and the spectral estimate for the second component.

5 84. The method of claim 68, wherein deriving the spectral estimate of the pure spectrum for the first component comprises adjusting values corresponding to the spectrally resolved information for the first set of spatial locations to remove a contribution from the second component based on the spectral estimate for the second component.

10 85. The method of claim 84, wherein the removed contribution is a maximal contribution.

86. The method of claim 85, wherein the maximal contribution is based on an error function analysis of the signal in each spectral channel of the adjusted values.

87. The method of claim 86, wherein the error function analysis tends to maintain nonnegative signal in each spectral channel of the adjusted values.

20 88. The method of claim 84, wherein the values comprises a series of at least some of the values for each of the spatial locations in the first set, and wherein removing the contribution from the second component based on the spectral estimate for the second component comprises subtracting an optimized quantity of the spectral estimate for the second component from each of the series of values.

25 89. The method of claim 88, wherein determining the optimized quantity for at least a first of the series of values is based on minimizing an error function of a difference spectrum that includes a difference between the first series values and the quantity to be optimized multiplied by the spectral estimate for the second component, wherein the error
30 function is minimized over the first series of values.

90. The method of claim 89, wherein the difference spectrum further includes a constant that is also optimized over the first series of values.

91. The method of claim 89, wherein the error function favors positive values of the difference spectrum over negative values of the difference spectrum.

92. The method of claim 91, wherein the error function comprises $(e^{-\Delta} + 1)\Delta^2$,
5 where Δ is the difference spectrum.

93. The method of claim 89, wherein the error function is normalized by the magnitudes of the first series of values and the spectral estimate for the second component.

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94. The method of claim 68, wherein the decomposition comprises a first decomposition of the spectrally resolved information at multiple spatial locations into contributions from initial spectral estimates associated with at least some of the components in the sample, improving an accuracy of at least some of the initial spectral
15 estimates based on the first decomposition, and at least a second decomposition of the spectrally resolved information at multiple spatial locations into contributions from the improved spectral estimates.

95. The method of claim 68, wherein the spectrally resolved information
20 comprises information about a set of images in which the light coming from the sample is spectrally filtered, wherein the spectral filtering for each image corresponds to a different spectral weighting function.

96. The method of claim 68, wherein the spectrally resolved information
25 comprises information about a set of images in which light used to illuminate the sample is spectrally filtered, wherein the spectral filtering for each image corresponds to a different spectral weighting function.

97. The method of claims 95 or 96, wherein the different spatial locations
30 correspond to common pixels in the set of images.

98. The method of claims 95 or 96, wherein the different spectral weighting functions correspond to different spectral bands.

99. The method of claims 95 or 96, wherein the set of images comprises three or more images.

100. The method of claims 95 or 96, wherein the set of images comprises four
5 or more images.

101. The method of claim 97, wherein the information about the set of images comprises a series of values at each of the pixels, wherein each value is related to an intensity of the light coming from the sample with respect to a corresponding one of the
10 spectral weighting functions.

102. The method of claim 68, wherein the spectrally resolved information for each spatial location comprises information corresponding to at least three different spectral weighting functions.

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103. The method of claim 68, wherein the spectrally resolved information for each spatial location comprises information corresponding to at least four different spectral weighting functions.

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104. The method of claim 68, wherein the spectrally resolved information comprises a spectral image cube.

105. The method of claim 68, wherein the light coming from the sample comprises fluorescence from the sample.

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106. The method of claim 68, wherein the light coming from the sample comprises reflectance, phosphorescence, scattering, or Raman scattering from the sample.

107. The method of claim 68, wherein the light coming from the sample
30 comprises transmission through the sample.

108. The method of claim 68, wherein at least one of the components relates to autofluorescence.

109. The method of claim 68, wherein at least one of the components comprises a target compound.

110. The method of claim 109, wherein the selected component is the
5 component comprising the target compound.

111. The method of claim 109, wherein the target compound comprises a fluorescent protein or a quantum dot.

112. The method of claim 68, further comprising illuminating the sample and
10 collecting the spectrally resolved information.

113. The method of claim 68, wherein collecting the spectrally resolved information comprises using a liquid crystal tunable spectral filter, an acousto-optical
15 tunable spectral filter, a set of spectral filters, a spectral filter wheel, a dispersive prism, a grating, a spectrometer, or monochromator.

114. The method of claim 68, wherein the image of the selected component comprises an image in which signal from the other components is reduced relative to
20 signal from the selected component.

115. The method of claim 68, further comprising constructing a second image of the sample based on the decomposition to preferentially show a second one of the components.
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116. An apparatus comprising:
a sample holder configured to support a deep tissue sample;
an illumination source to illuminate the sample;
30 a detector positioned to detect light from the sample; and
an electronic processor coupled to the detector, wherein the electronic processor and is configured to: (i) provide spectrally resolved information about light coming from different spatial locations of a sample, wherein the light includes contributions from different components in the sample; (ii) decompose the spectrally resolved information

for each of at least some of the different spatial locations into contributions from spectral estimates associated with at least some of the components in the sample; and (iii) construct a deep tissue image of the sample based on the decomposition to preferentially show a selected one of the components.

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117. An apparatus comprising:

a sample holder configured to support a deep tissue sample;

an illumination source to illuminate the sample;

a detector positioned to detect light from the sample; and

10 an electronic processor coupled to the detector, wherein the electronic processor and is configured to: (i) provide spectrally resolved information about light coming from different spatial locations of a sample, wherein the light includes contributions from different components in the sample and the spectrally resolved information for each spatial location comprises information corresponding to at least three different spectral
15 weighting functions; and (ii) construct a deep tissue image of the sample based on the spectrally resolved information to preferentially show a selected one of the components.

118. An apparatus comprising:

a sample holder configured to support a sample;

20 an illumination source to illuminate the sample;

a detector positioned to detect light from the sample; and

an electronic processor coupled to the detector, wherein the electronic processor and is configured to: (i) provide spectrally resolved information about light coming from different spatial locations of a sample, wherein the light includes contributions from
25 different components in the sample; (ii) decompose the spectrally resolved information for each of at least some of the different spatial locations into a contribution from a spectral estimate of a pure spectrum for at least a first one of the components in the sample; and (iii) construct an image of the sample based on the decomposition to preferentially show a selected one of the components, wherein the decomposition
30 comprises deriving the estimate of the pure spectrum for the first component based on the spectrally resolved information corresponding to a first set of one or more of the different spatial locations and a spectral estimate of a pure spectrum for a second one of the components.

119. The apparatus of claims 116, 117, or 118, further comprising a spectral filtering means positioned between the sample and the detector.

120. The apparatus of claim 119, wherein the spectral filtering means comprises
5 a liquid crystal tunable spectral filter, an acousto-optical tunable spectral filter, a set of spectral filters, a spectral filter wheel, a dispersive prism, a grating, a spectrometer, or monochromator.

121. The apparatus of claims 116, 117, or 118, further comprising a spectral
10 filtering means positioned between the illumination source and the sample.

122. The apparatus of claims 116, 117, or 118, wherein the illumination source provides tunable excitation light.

123. Apparatus comprising a computer-readable medium storing a program that
15 causes a processor to carry out the steps of any of claims 1, 60, and 68.

124. A method comprising:
illuminating a sample to cause the sample to emit radiation, wherein the
20 sample comprises deep tissue supporting a component comprising a target compound, and wherein the emitted radiation comprises emission from the target compound and emission from one or more other components in the sample,
spectrally filtering the emitted radiation with each of a plurality of
different spectral weighting functions;
25 storing an image of the spectrally filtered radiation for each of the spectral weighting functions; and
processing the stored images to construct a deep tissue image of the sample in which signal from the additional components is reduced relative to signal from the target compound.

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125. A method comprising:
illuminating a sample to cause the sample to emit radiation, wherein the sample
comprises deep tissue supporting a component comprising a target compound, and

wherein the emitted radiation comprises emission from the target compound and emission from one or more other components in the sample,

spectrally filtering the emitted radiation with each of a plurality of different spectral weighting functions;

5 storing an image of the spectrally filtered radiation for each of the spectral weighting functions; and

processing the stored images to construct a deep tissue image of the sample in which signal from the additional components is reduced relative to signal from the target compound.

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126. The method of claim 125, wherein the sample comprising the deep tissue is a living organism.

127. The method of claim 126, wherein the living organism is a mammal.

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128. The method of claim 127, wherein the mammal comprises a mouse or a human.

129. The method of claim 126, wherein the living organism is a zebrafish.

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130. The method of claim 126, wherein the deep tissue is an internal organ of the living organism.

131. The method of claim 126, wherein the deep tissue lies within about 2 mm or more of the living organism.

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132. The method of claim 125, wherein the deep tissue is a subdermal tissue.

133. The method of claim 125, wherein the emission from the other components of the sample comprises autofluorescence from tissue overlying the deep tissue.

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134. The method of claim 125, wherein the emission from the other components of the sample comprises autofluorescence from one or more layers of tissue in the sample different from a layer of tissue comprising the deep tissue.

5 135. The method of claim 125, wherein the target compound is a fluorescent probe bound to at least a portion of the deep tissue.

136. The method of claim 125, wherein the target compound is a luminous probe bound to at least a portion of the deep tissue.

10 137. The method of claim 125, wherein the target compound is a quantum dot bound to at least a portion of the deep tissue.

138. The method of claim 125, wherein the target compound comprises deep
15 tissue portions expressed by green fluorescent protein (GFP).

139. The method of claim 125, wherein the target compound comprises deep tissue portions expressed by yellow fluorescent protein (YFP).

20 140. The method of claim 125, wherein the target compound comprises deep tissue portions expressed by red fluorescent protein (RFP).

141. The method of claim 125, wherein the emission from the target compound is fluorescence.

25 142. The method of claim 125, wherein at least some of the spectral weighting functions correspond to particular wavelength bands.

143. The method of claim 17, wherein all of the spectral weighting functions
30 correspond to particular wavelength bands.

144. The method of claim 125, wherein at least some of the spectral weighting functions correspond to sinusoidal weightings of multiple wavelength bands.

145. The method of claim 125, wherein the spectral filtering comprises using a liquid-crystal, tunable optical filter.

146. The method of claim 125, wherein the spectral filtering comprises using an
5 interferometric optical filter.

147. The method of claim 125, wherein the spectral filtering comprises using a filter wheel containing a plurality of band pass filters.

10 148. The method of claim 125, wherein each stored image comprises an intensity value for each of multiple pixels.

149. The method of claim 125, wherein processing the stored images comprises constructing the deep tissue image based on a weighted superposition of signal in the
15 stored images.

150. The method of claim 125, wherein processing the recorded images comprises constructing the deep tissue image based on the recorded images and at least one emission spectrum for the other components in the sample.

20 151. The method of claim 150, wherein constructing the deep tissue image comprises calculating a remainder spectrum for each pixel in the set of stored images.

152. The method of claim 150, wherein processing the recorded images
25 comprises constructing the deep tissue image based on the recorded images, the at least one emission spectrum for the other components in the sample, and an emission spectrum for the target compound.

153. The method of claim 152, wherein constructing the deep tissue image
30 comprises solving at least one component of a matrix equation in which one matrix is based on the stored images, and another matrix is based on the emission spectra.

154. The method of claim 125, wherein processing the recorded images comprises constructing the deep tissue image based on the recorded images and an emission spectrum for the target compound.

5 155. The method of claim 154, wherein constructing the deep tissue image comprises calculating a remainder spectrum for each pixel in the set of stored images.

156. The method of claim 125, wherein the deep tissue supports multiple target compounds and processing the stored images comprises constructing a deep tissue image
10 for each of the target compounds.

157. The method of claim 156, wherein processing the recorded images comprises constructing the deep tissue images based on the recorded images and emission spectra for the target compounds.

158. The method of claim 157, wherein processing the recorded images comprises constructing the deep tissue images based on the recorded images, the emission spectra for the target compounds, and at least one emission spectrum for the other components in the sample.

159. The method of claim 125, wherein the plurality of the different spectral weighting functions comprises at least four spectral weighting functions.

160. A method comprising:
25 providing a plurality of images of spectrally filtered radiation emitted from a sample in response to an illumination,
wherein the sample comprises deep tissue supporting a target compound,
wherein the emitted radiation comprises emission from the target compound and emission from one or more other components in the sample, and
30 wherein each image corresponds to a different spectral weighting function; and
processing the images of the spectrally filtered radiation to construct a deep tissue image of the sample in which signal from the other compounds is reduced relative to signal from the target compound.

161. Apparatus comprising a computer readable medium which stores a program that causes a processor to:

receive a plurality of images of spectrally filtered radiation emitted from a sample in response to an illumination,

5 wherein the sample comprises deep tissue supporting a target compound, wherein the emitted radiation comprises emission from the target compound and emission from one or more other components in the sample, and wherein each image corresponds to a different spectral weighting function; and process the images of the spectrally filtered radiation to construct a deep tissue

10 image of the sample in which signal from the other compounds is reduced relative to signal from the target compound.

162. An apparatus comprising:

a sample holder configured to hold a sample comprising deep tissue, wherein the

15 deep tissue supports a target compound;

an illumination source configured to illuminate the sample to cause it to emit radiation, wherein the emitted radiation comprises emission from the target compound and emission from one or more other components in the sample;

an imaging system configured to image the emitted radiation to a detector;

20 a tunable spectral filter configured to spectrally filter the emitted radiation with each of a plurality of different spectral weighting functions;

a detector configured to store an image of the spectrally filtered radiation for each of the spectral weighting functions; and

a electronic processor configured to process the store images to construct a deep

25 tissue image of the sample in which signal from the other compounds is reduced relative to signal from the target compound.

163. The apparatus of claim 162, wherein the sample holder is configured to hold an animal.

164. The apparatus of claim 162, wherein the imaging system has a demagnification greater than or equal to 1.

165. The apparatus of claim 162, wherein the imaging system is configured to image a field of view having a diagonal dimension greater than about 2 cm onto the detector.

5 166. A method comprising:

providing a set of images of spectrally filtered radiation emitted from a biological sample in response to an illumination, wherein the sample comprises a component supporting a target compound, the emitted radiation comprises emission from the target compound and emission from one or more additional components in the sample, and each
10 image corresponds to a different spectral weighting function for a common set of pixels; and

processing the images of the spectrally filtered radiation to construct an output image of the sample in which signal from the additional components is reduced relative to signal from the target compound,

15 wherein the processing comprises calculating a remainder spectrum for one or more pixels in the set of images based on an estimate for an emission spectrum of at least one of the components.